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TRPM2: a potential drug target to retard oxidative stress

Jun Li[1, 4, †], Yunling Gao[2, †], Xianying Bao[1, 3], Fengna Li[1], Wei Yao[5], Zemeng Feng [1, 6, *], Yulong Yin[1]

[1]Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences; National Engineering Laboratory for Pollution Control and Waste Utilization in Livestock and Poultry Production; Hunan Provincial Engineering Research Center for Healthy Livestock and Poultry Production; Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South-Central, Ministry of Agriculture, Changsha, Hunan 410125, Peoples R China, [2]Xiangyang Central Hospital, the Affliated Hospital of Hubei University of Arts and Science, Xiangyang, 441021, Peoples R China, [3]College of Animal Science and Technology, Hunan Agricultural University, Changsha, 410128, China, [4]University of Chinese Academy of Sciences, Beijing 100049, Peoples R China, [5]Department of Cardiology, Tianjin Medical University General Hospital, Tianjin Medial University, Tianjin 300052, China, [6]Hunan Co-Innovation Center of Animal Production Safety, CICAPS, Changsha, 410128, Peoples R China

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1. ABSTRACT

The **T**ransient **R**eceptor **P**otential **M**elastatin 2 (TRPM2) is a member of G protein coupled receptor superfamily and a novel dual-function protein that possesses both ion channel and **A**denosine 5'-**D**iphos-**P**hatase **R**ibose (ADPR) hydrolase function. TRPM2 is involved in Ca^{2+} signaling in various cells as an endogenous redox sensor for oxidative stress and reactive oxygen species, and contributes to cytokine production, insulin release, motility, Ca^{2+} entry and Ca^{2+}-dependent cellular reactions such as endothelial hyper-permeability and apoptosis. The wide expression of TRPM2 might render it as a potentially significant therapeutic target in pathological settings including cardiovascular and neurodegenerative diseases and of great relevance in drug design, feed additives and other industries. Here, we discuss the TRPM2 gene structure, function, its variants, as well as its activators and inhibitors and provide a peptide drug design for modulation of oxidative stress.

2. INTRODUCTION

Oxidative **S**tress (OS) represents an imbalance between the generation of **R**eactive **O**xygen **S**pecies (ROS) and the functions of antioxidant systems (1). Disturbances in the normal redox state of tissues will induce toxic effects through ROS that could damage proteins, lipids, DNA and other molecules (2). Suitable concentrations of ROS are beneficial and, in fact, necessary in lives due to their signaling roles in metabolism (3). However, when under various stresses, like cold, heat, age, disease, and other conditions, the balance between ROS production and quenching is disturbed, OS is induced and bring damages to organisms, and further induce sickle cell disease, atherosclerosis, parkinson's disease, **A**lzheimer's **D**isease (AD), heart failure, myocardial infarction, Schizophrenia, **B**ipolar **D**isorder (BD), fragile X syndrome and chronic fatigue syndrome (4-6).

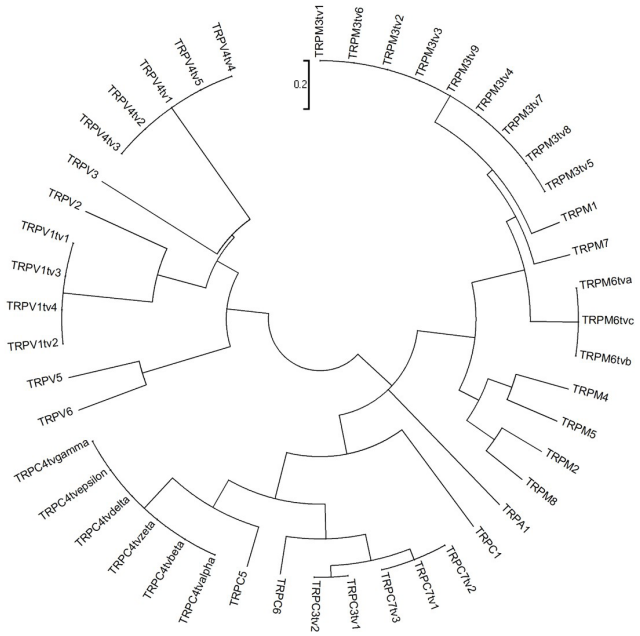

Figure 1. Phylogenetic tree for *Homo sapiens* (human) TRP channels built by using MEGA 4.1, tv means transcript variant.

The **T**ransient **R**eceptor **P**otential **M**elastatin 2 (TRPM2) is a member of G protein coupled receptor superfamily and a novel dual-function protein that possesses both ion channel and **A**denosine 5'-**D**iphos-**P**hatase **R**ibose (ADPR) hydrolase function, and the two functions are uncoupled (7-9). It plays a pivotal role in temperature sensing, cancer, numerous metabolic and neuronal diseases such as diabetes mellitus, BD and AD (10-15). TRPM2 also acts as an endogenous redox sensor for mediating OS / ROS-induced Ca^{2+} entry and the subsequent specific Ca^{2+}-dependent cellular reactions. As for the nearly half of commercial medicines target to G protein coupled receptor superfamily, TRPM2 is also a potentially significant therapeutic target for diseases, and researches on it can be of great relevance in drug designing, feed additives and other industries (16).

3. THE GENE LOCATION, TISSUES DISTRIBUTION OF TRPM2

The TRPM2 gene was initially reported as transient receptor potential related channel 7 by the genomic DNA sequence analysis (Figure 1). This gene consists of 32 exons and spans approx. 90 kb (17). In human, it locates on disease-rich chromosome 21q22.3, pitches in the region between two markers, D21S400 and D21S171 (18, 19). Chromosome 21q22.3. (Figure 2) is near to the SOD1 locus (21q22.1.–22.2.), which is associated with familial amyotrophic lateral sclerosis (20).

TRPM2 is predominantly expressed in brain (17), and to be highest in the hippocampus (21), cerebral cortex, thalamus, and midbrain, specifically in microglia and neuronal cell (22). TRPM2 is also detected in many other tissues, including bone, gastrointestinal tract, marrow, spleen, heart, liver, pancreas, placenta, ovary, lung, vascular smooth muscle, hematopoietic cells, monocytes, endocrine cells and endothelial cells (23).

4. THE BASIC STRUCTURE OF TRPM2

TRPM2 comprise an ion channel core domain and cytoplasmic NH_2 and COOH termini. Similar to other six **T**rans-**M**embrane (6TM) channels including cyclic nucleotide-gated channels, its ion channel domain (residues 762-1048) is composed of 6TM segments (S1-S6) and a pore loop between S5 and S6 (Figure 3). The distal part of S6 is important for the gating function for charge discrimination (24). No functional role be attributed to any of the structural motifs within the N-stretch that may be a spacer segment for other functional sites in the N terminus (25). Glu-960, Gln-981, Asp-987, and Glu-1022 residues are engaged in determining divalent cationic permeation properties of the channel. The residues Glu-960, Gln-981, and Asp-987 contribute significantly to defining Ca^{2+} permeation while Glu-1022 to Mg^{2+} (26). The N-terminal cytosolic tail of about 700 amino acids contains calmodulin binding domains that mediate Ca^{2+} regulation of TRPM2 activity (25). The N-terminal part also has a high affinity binding site for **P**rotein **K**i-nase **C**-alpha (PKCα) and two PxxP motifs implicated in protein-protein interactions, so this part likely to be crucial for the proper interaction with essential regulatory cytosolic components, assembly of the

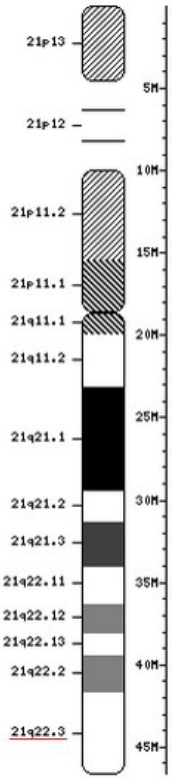

Figure 2. The master map of *Homo sapiens* (human) Chromosome 21. Region located TRPM2 was point out with red underline.

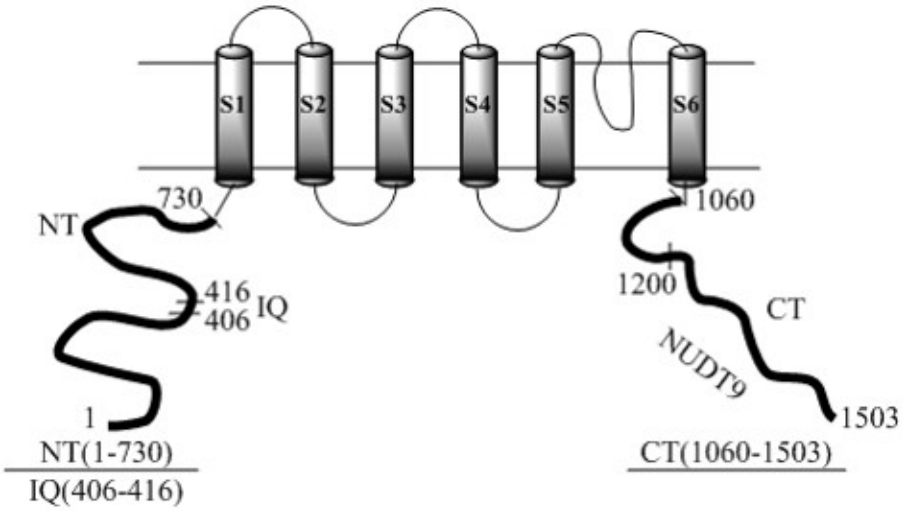

Figure 3. Diagram of TRPM2. The N- and C-terminal fragments (dark lines), the IQ-like motif (residues 406-416), the NUDT9 ADPR hydrolase domain (residues 1236-1503), and the six transmembrane domains (S1-S6) are shown.

channel units and membrane trafficking (25). There are two coiled-coil domains contained in TRPM2. The one in N-terminal part (655-679) is required for protein expression and function, but not subunit interaction, and the Ile-658 residue in this domain is required for normal channel function (27), while the other one in the C-terminus may play a role in ion channel subunit multi-mediation or in recruitment of regulatory proteins (28). The whole TRPM subfamily contains a pair of cysteine residues (positions 996 and 1008 in the human TRPM2 subunit), and the first is completely conserved (Figure 4) while the second is present at slightly different positions. The two cysteine residues and Lys[1110] are obligatory for TRPM2 channel function

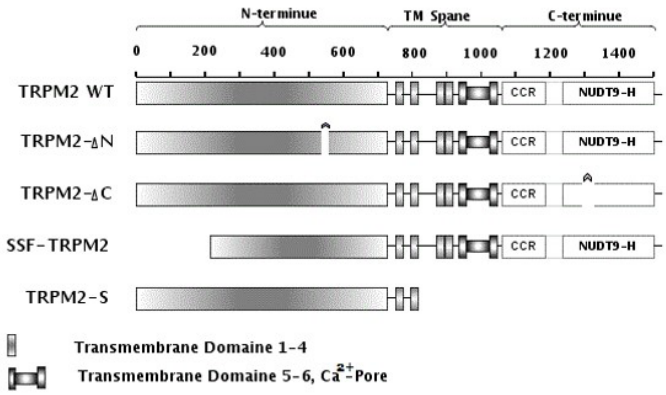

Figure 4. Schematic representation of TRPM2 isoforms. Membrane spanning domains 1-4 and the putative pore region including transmembrane domains 5-6 are indicated. CCR represents the coiled coil region which may mediate protein/protein interactions. NUDT9-H represents the NUDT9 ADPR hydrolase domain. TRPM2-DN has a deletion of amino acids 538-557 in the N-terminus. TRPM2-DC has a deletion of AA 1292-1325 in the C-terminus. TRPM2-S is missing four of six transmembrane domains and the putative calcium pore.

(29, 30). Functional TRPM2 molecules are tetramers and subunit composition is a factor in regulation of the channel opening (31).

5. VARIANTS OF TRPM2

Until now, some TRPM2 variants have been found, each of them has unique construction and characters, which was showed in Figure 4. TRPM2-S was cloned from human bone marrow and consists of only the N terminus and the first two trans-membrane domains. The four C-terminal trans-membrane domains, the putative calcium-permeable pore region, and the entire C terminus were deleted because of a stop codon (TAG) alternative splicing which locates at the splice junction between exons 16 and 17. Interaction between TRPM2-S and TRPM2-WT is an important mechanism for channel activity regulation and cellular response to OS (32). Expression of TRPM2-S inhibited susceptibility to cell death and onset of apoptosis induced by H_2O_2 in cells expressing TRPM2-WT.

The striatum short form (SSF-TRPM2), detected only in striatum (caudate nucleus and putamen), is a 5.5 kb shorter transcript transcribed from the intron 4 of the TRPM2 gene with 1289 amino acid residues. As compared to the long form protein, the N-terminal 214 amino acid residues are removed in SSF-TRPM2. The SSF-TRPM2 still maintained H_2O_2-induced Ca^{2+} influx activity (19).

TRPM2ΔNΔC is a new TRPM2 isoform by two deletions, from 538 to 557 and from 1292 to 1325. Additionally, two amino acid residues are exchanged (S1088N and D1291E). One deletion is located in the cytosolic N terminus and the other one in the cytosolic C terminus of TRPM2. This splicing variant lost channel activity to respond to ADPR (33).

TRPM2P1018L produces a missense change whereby proline 1018 (Pro1018) is replaced by leucine (Leu1018), and inactive the channel function. The ability of TRPM2 to maintain sustained ion influx is a physiologically important function and that its disruption may, under certain conditions, contribute to disease states. TRPM2P1018L may confer susceptibility to western pacific amyotrophic lateral sclerosis and parkinsonism-dementia, which shared a unique mineral environment characterized by the presence of severely low levels of Ca^{2+} and Mg^{2+}, coupled with high levels of bioavailable transition metals in the soil and drinking water (34). TRPM2 genetic variants also have significant association with BD. TRPM2 SNP rs1556314 in exon 11 was significantly associated with BD-I but not BD-II (35).

6. ACTIVATORS FOR TRPM2

ADPR is the intracellular TRPM2 agonist (36). However, ADPR alone is insufficient to gate TRPM2 channel rather channel activation is contingent on the binding of Ca^{2+} to an intracellular channel domain via calmodulin (37, 38). ADPR most likely binds to C-terminal Nudix hydrolase domain NUDT9H for TRPM2 activation (Figure 5), but there also may be other mechanism (39). TRPM2 channel is intimately coupled to N-methyl-d-aspartate receptor s via Ca^{2+} influx (40)

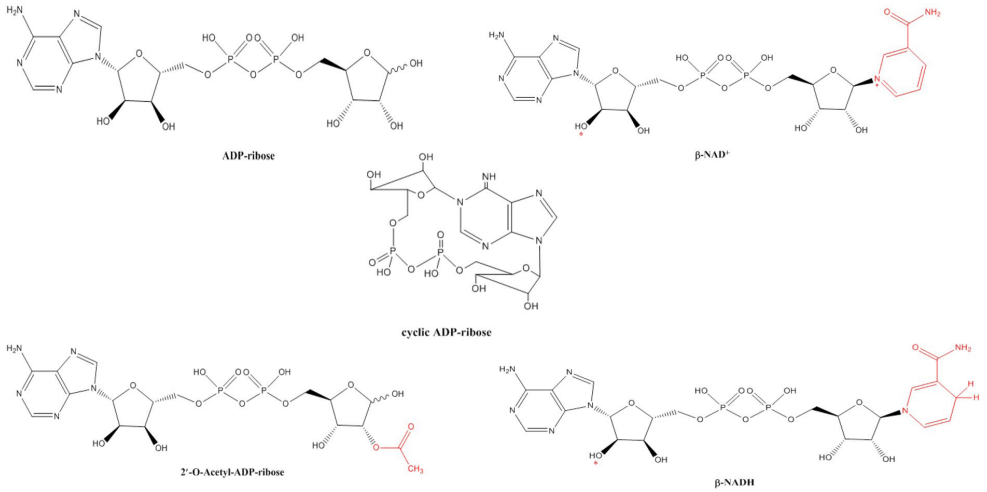

Figure 5. Molecular structures of ADPR and mainly related adenine nucleotides that can activate TRPM2 channels.

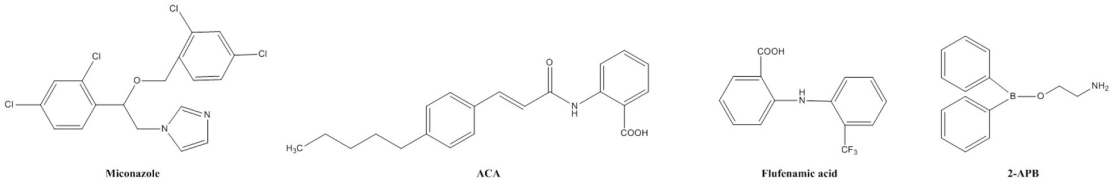

Figure 6. Molecular structures of mainly TRPM2 channel inhibitors.

and are necessary for the induction of N-methyl-d-aspartate receptor -dependent long term depression (41). Additionally, nicotinic acid adenine dinucleotide phosphate, cyclic ADPR, 2'-O-acetyl-ADPR, ADPR-2'-phosphate and H_2O_2 facilitate activation of TRPM2 channel (33, 42-45). The mechanism of how H_2O_2 activate TRPM2 is still unclear. Hara *et al* reported that TRPM2 activation by H_2O_2 is mediated by increase of NAD^+ levels and binding of NAD^+ to the nudix domain, but Kühn and Lückhoff thought the channels are not gated by H_2O_2, but NAD^+ (46, 47). Others thought H_2O_2 activates TRPM2 through the release of ADPR from the mitochondria or the ectoenzyme CD38 (42, 48). Conversely, the direct activation of TRPM2 by cyclic ADPR and nicotinic acid adenine dinucleotide phosphate, albeit limited, cannot be suppressed by the specific ADPR antagonist AMP (42). The intracellular Ca^{2+} appears to be an important modulator and cofactor of TRPM2, as elevated intracellular Ca^{2+} can significantly increase the sensitivity of TRPM2 toward ADPR (46). TRPM2 channel activation is sensitive to intracellular Cl^- concentration (49).

7. INHIBITORS FOR TRPM2

Generally, there are less specific TRPM2 inhibitors which have a potentially clinically useful effect on the activation are found now. But several compounds show inhibit ability toward TRPM2, they are probably divided into 2 different classes, namely the fenamates, such as flufenamic acid (FFA), and anti-fungal imidazoles (miconazole, econazol and clotrimazole) (50, 51) (Figure 6). FFA, a nonsteroidal anti-inflammatory drug, is a pH-dependent antagonist of TRPM2 channels. Decreasing extracellular pH accelerated FFA inhibition of TRPM2. FFA is not easily dissolved in the aqueous solution, which inhibit its application (50). N- (p-amylcinnamoyl) anthranilic acid (ACA), usually functions as a phospholipase A_2 inhibitor, is another inhibitor of TRPM2 (52), it modulates different TRP channels most probably by a direct interaction. ACA induces a complete and rapid decline of TRPM2 currents in cells overexpressing this channel, most likely by interfering with the pore and in a manner independent of the channel activator (24, 52). Owing to its high potency and efficacy on TRPM2, ACA can serve, in combination with other blockers, as a pharmacological tool for studying H_2O_2-induced Ca^{2+} signal and biological functions of TRPM2 channels in native cells. Chlorpromazine, Curcumin and N-Acetylcysteine also inhibits activation of TRPM2 channels (53-55). 8- phenyl-2'-deoxy-ADPR can highly specific binding to NUDT9H and inhibit TRPM2 (56).

Although FFA, miconazole and clotrimazole, and ACA inhibit TRPM2, their inhibition was either gradual or irreversible. For example imidazole derivatives, miconazole and clotrimazole, inhibit

TRPM2 in an irreversible manner (51). 2-aminoethyl diphenylborinate (2-APB) can reversibly and completely inhibit TRPM2 (57).

TRPM2 is also inhibited by both intracellular and extracellular acidic pH. Extracellular protons inhibit TRPM2 by decreasing single-channel conductance. H958, D964, and E994, at the outer vestibule of the channel pore are responsible for this characteristic. Titration of H958, D964, and E994 by external protons inhibits TRPM2 gating by causing conformation change of the channel, and/or by decreasing local Ca^{2+} concentration at the outer vestibule, therefore reducing Ca^{2+} permeation and inhibiting Ca^{2+} -mediated TRPM2 gating. Intracellular protons inhibit TRPM2 by inducing channel closure without changing channel conductance. D933 located at the C terminus of the S4-S5 linker is responsible for intracellular pH sensitivity. D933 is not only essential for intracellular pH sensitivity, but it is also crucial for TRPM2 channel gating (58).

8. TRPM2 WILL BE ACTIVATED DURING OXIDATIVE STRESS

TRPM2 is shown to gate in response to OS via second-messenger production (59), which can be inhibited by pharmacological reagents. Oxidants, applied externally or produced in the cytosol during OS (33), stimulate ADPR formation in the nucleus and mitochondria (48). The free radicals intermediates produced in the cytosol during OS include superoxide anion (O^{2-}), H_2O_2, nitric oxide, and a more damaging compound, hydroxyl radical (OH.). These radicals contribute to DNA oxidation and damage, which initiate PARP-mediated ADPR generation. PARP act as a potential source of ADPR for the activation of TRPM2 in OS. PARP binds to single-stranded and double-stranded DNA breaks and catalyses the breakdown of NAD into nicotinamide and poly (ADPR) to initiate DNA repair mechanisms (48). Free ADPR is then generated from poly (ADPR) degradation by poly (ADPR) glycohydrolase. It has been suggested that OS-induced TRPM2 activation is triggered via the production of ADPR from mitochondria (48). TRPM2 confers susceptibility to cell death through the activation of caspases and PARP (46). Alternative supply of ADPR may results from the direct hydrolysis of NAD^+ into nicotinamide and ADPR or indirectly through cyclic ADPR formation. This reaction is catalyzed by NADases that are not only located at cell surface, but also in mitochondria and the nucleus (60). Although most investigators demonstrated an indirect action of oxidants on TRPM2 through ADPR generation, direct action of oxidants on TRPM2 has been also proposed for neutrophil granulocytes, as the TRPM2-ΔC splice variant was stimulated by H_2O_2 but did not respond to ADPR (33). H_2O_2-mediated Ca^{2+} entry through TRPM2 in pulmonary artery endothelial cells was reduced by at least 65 % in cells treated with a PARP inhibitor (either 3, 4-dihydro-5- (4- (1-piperidinyl)butoxyl)-1 (2H)-isoquinolinone or 3-aminobenzamide) to prevent ADPR agonist formation (61). Phosphotyrosine phosphatase L1 and PKCα can regulate oxidant-induced TRPM2 activation (62). The H_2O_2 induced apoptotic changes involve TRPM2 opening, which results in mitochondrial $(Na^+)_m$ (and $(Ca^{2+})_m$) overload, followed by mitochondrial membrane disruption, cytC release, and caspase 3-dependent nuclear condensation /fragmentation, while the necrotic changes are caspase-3-independent, but PARP dependent. Inhibition of both TRPM2 and PARP activities totally abolishes H_2O_2-induced myocyte death (23).

9. FUNCTION AND POTENTIAL APPLICATION IN DRUG DISCOVERY OF TRPM2

TRPM2 regulates endothelial barrier function, plays a critical role in the mechanism of endothelial barrier disruption following OS (63), inhibition of TRPM2 may provide a useful therapeutic strategy for the treatment of endothelial barrier dysfunction and vascular inflammation (61). TRPM2 is abundant in pancreatic islet cells (64) and plays a critical role in insulin secretion by pancreatic β-cells. TRPM2 also functions as a Ca^{2+}- release channel activated by intracellular ADPR in a lysosomal compartment. TRPM2-mediated chemokine production in monocytes/macrophages is an important mechanism in the progressive severity of DSS-induced ulcerative colitis. Ca^{2+} influx via native TRPM2 plays an important role in the H_2O_2-induced CXCL8 production inmonocytes. Erk and NF-ĸB are involved in CXCL8 production inmonocytes and other cell types (65). Erk activation amplified by Ca^{2+} influx through TRPM2 is mediated *via* Ras (66). TRPM2 are potential therapeutic targets for oxytocin release in psychiatric diseases caused by social stress (67).

10. DISCUSSION

TRPM2 is implicated in endothelial dysfunction and many pathological states, which has emerged as an important Ca^{2+} signal molecular in various cells, and be involved in cytokine production, insulin release, motility and death. It is a potentially significant therapeutic target in pathological settings including cardiovascular and neurodegenerative diseases (68).

Chemical drug occupied most of modern drugs, which are susceptibility to resistance and usually have long-term toxicity. The use of these drugs should be used under serious supervision. So, new safely and effectively therapeutic approaches were developed in clinic. Peptides have attractive features compared to small molecule and protein, such as high

structural compatibility with target protein, disrupted protein-protein interfaces, less susceptibility to drug resistance, small size, lower costs, improved organ or tumor penetration and higher activity per mass when compared to antibodies or large proteins (69, 70). Novel delivery and chemical modification technologies have sparked much interest in peptide therapeutics (71-73). More than 50 peptide-based products have been approved for clinical use (e.g. Fuzeon (Roche); Byetta (Amylin/Eli Lilly); Sandostatin (Novartis); Zoladex (Astra- Zeneca); Copaxone (Teva)) (74). Peptide-protein interactions have important roles in mediating in protein-protein interactions, predominantly in signaling and regulatory networks (75), which are also attractive drug targets for designed inhibitory peptides (76). As for peptide drug design, there been some strategies, such as peptide phage display (77). Researchers described computational framework to identify peptide ligands to membrane channel receptors by producing sequence alignments across many species at the functional-element level (78).

Its ability to respond to ROS has made TRPM2 a potential therapeutic target for chronic inflammation, neurodegenerative diseases, and OS-related pathologies. The physiological and pathophysiological context of ROS-mediated events makes TRPM2 a promising target for the development of therapeutic tools of inflammatory and degenerative diseases. In fact, because OS contributes to numbers of pathophysiological conditions, it is meaningful to investigate TRPM2, because it may be an important potential target to discover new drugs to cure diseases with aging especially neurologic disease. Though the mechanisms of TRPM2 activation and regulation has gained significant interest, the relationship between channel activation, intracellular Ca^{2+} rise and cell death is still unclear (68), a problem compounded by uncertainty of the second messengers involved and the limited specificity of the pharmacological blockers, then it's very imperative to find or design a new drug to regulate the channel and to do advance research. Peptide omimetics is the approach of reproducing the biological activity or binding properties in a smaller molecule, like peptides or modified peptides which were designed to mimic the desired region. The structural basis of peptide-protein binding strategies also analysis and found most peptides do not induce conformational changes on their partner upon binding, thus minimizing the entropic cost of binding. Peptides display interfaces that are better packed than protein-protein interfaces and contain significantly more hydrogen bonds, mainly those involving the peptide backbone. Additionally, ''hot spot'' residues contribute most of the binding energy (79), and these residues can be accurately predict (80). Finally, peptides tend to bind in the largest pockets available on the protein surface (79). Peptides designed to mimic the VEGF binding site to its receptor VEGFR-2 has shown effective function to inhibit endothelial cell proliferation, migration and network formation (81). As far as concerned TRPM2, peptides could be design similar to or peculiar binding to the NUDT9-H domain, which could inhibit or activate TRPM2 channel gating. As peptides cannot last for a long time, the designed peptide drugs has efficacy neither gradual nor irreversible, but reversible and can be controlled through dose and administration time.

11. ACKNOWLEDGEMENT

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Abbreviations: 2-APB: 2-aminoethoxydiphenyl borate; 6TM: Six trans-membrane; ACA: N-(p-amylcinnamoyl) anthranilic acid; ADPR: Adenosine diphospho-ribose; AMP: adenosine monophosphate; BD: Bipolar disorder; FFA: flufenamic acid; H2O2: hydrogen peroxide; NAD: nicotinamide adenine dinucleotide; NADases: NAD+ glycohydrolases; NUDT9-H: Nucleoside diphosphate linked moiety X-type motif 9 homology; O2: Oxygen; PARP: poly-adenine dinucleotide phosphate-ribose polymerase; ROS: Reactive oxygen species; SNP: single nucleotide polymorphisms; SOD: superoxide dismutase ; SSF-TRPM2: striatum short form transient receptor potential (melastatin) 2; TNFα: tumor necrosis factor α; TRP: The transient receptor potential channel; TRPC7: Transient receptor potential-related channel 7; TRPM2: The transient receptor potential (melastatin) 2; TRPM2-WT: The transient receptor potential (melastatin) 2 wild type.

Key Words: Transient receptor potential melastatin 2, Oxidative stress, Calcium-permeable channel, Drug design, Review

Send correspondence to: Zemeng Feng, Address: Mapoling of Changsha City, Hunan province, P. R. China, Tel: +86-731-84619706, Fax: 86-731-84612685, E-mail: zemengfeng2006@163.com